CHYTRIDS IN EGYPT: II - NEW RECORDS OF SPECIES OF ENTOPHLYCTIDACEAE

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ABSTRACT - Eight newly recorded species of the Entophlyctidaceae, *Endochytrium digitatum* Karling, *Entophlyctis crenata* Karling, *E. vaucheriae* Fischer, *Diplophlyctis nephrochytrioides* Karling, *D. sexualis* Haskins, *D. verrucosa* Kobayaschi, *Nephrochytrium amazonense* Karling and *N. appendiculatum* Karling were isolated and identified from water samples collected from different water streams located in El-Minia Governorate. Isolation and subculturing of these fungi were performed by using sterile cellulosic baits.

RÉSUMÉ - Huit espèces d'Entophlyctidacées nouvelles pour l'Egypte: *Endochytrium digitatum* Karling, *Entophlyctis crenata* Karling, *E. vaucheriae* Fischer, *Diplophlyctis nephrochytrioides* Karling, *D. sexualis* Haskins, *D. verrucosa* Kobayaschi, *Nephrochytrium amazonense* Karling et *N. appendiculatum* Karling, ont été isolées et identifiées à partir d'échantillons d'eau récoltés dans différentes rivières du governorat d'El-Minia. Ces champignons ont été isolés à l'aide de pièges cellulosiques stériles.

INTRODUCTION

Family Entophlyctidaceae are characterized by monocentric intramatrical thallus, composed of extramatrical zoospore cyst, intramatrical sporangia or resting spores and branched rhizoids. Whiffen (1944) divided this family into two subfamilies, Entophlyctoideae and Diplophlyctoideae, on the basis of whether the zoosporangia and resting spores develop directly from the germ tube or later from an apophysis. In the zoospores of *Entophlyctis* some authors, such as Koch (1968) and Salkin (1970) observed variations in spore size, number and size of lipid bodies.

In Egypt, there is no previous record or evidence of the occurrence of Entophlyctidaceae. The present studies were undertaken to enlarge our knowledge of the Egyptian zoosporic fungi. In this paper 8 species belonging to 4 genera in the Entophlyctidaceae are reported, isolated and identified from water streams in El-Minia Governorate.

MATERIALS AND METHODS

The materials and methods are the same as in the previous paper (Hassan, 1991).

RESULTS

All the strains isolated in the present study were recorded once and subcultured on the different cellulose substrates as shown in the materials and methods (Hassan, 1991).

A) Subfamily Entophlyctidioideae:

1 - *Endochytrium digitatum* Karling 1938 (Plate 1).

Thalli numerous, intramatrical, monocentric, eucarpic. Zoosporangia hyaline and smooth, elongate and obclavate 21-52 x 15-30μm, pyriform 25-140 x 15-53μm, obpyriform, irregular or subglobose and lobed, with one, rarely two simple or branched, curved or straight, tapering exit tubes, 56-98 x 4-10μm; operculum globose or slightly oval, 3-6μm diam. Zoospores hyaline, globose 4.2-5.6μm diam., with one refractive globule, posteriorly uniflagellate, emerging fully formed and singly, and resting in a globular mass a short while before becoming motile, intermittently amoeboid. This strain was baited with bromegrasse leaves.

The Egyptian isolate of *E. digitatum* fully matches the original description.

2 - *Entophlyctis crenata* Karling 1967 (Plate 2).

Sporangia intramatrical, single, subglobose or cylindrical with rounded ends, 32-98 x 25-70μm, terminal or intercalary, separated by cross walls from the rhizoidal system, with a single short papilla which penetrates the wall of the substratum, rhizoidal system dichotomously branched, polycentric, the main axis arising from one end or from opposite ends of the sporangium, broad and tubular, up to 5μm diam. Zoospores subglobose to slightly ovoid, 4-6μm diam., with a single globule and numerous small globules, posteriorly uniflagellate emerging upon deliquescence of the papilla and forming a temporary compact motionless mass at the orifice before assuming motility. Resting spores borne like sporangia, globose, up to 49μm diam., with a thickened colourless wall, 1.5-2μm thick. This strain was baited with bromegrasse leaves.

Most features of the Egyptian isolate of *E. crenata* match the type except for the resting spores which have a thinner wall and are crenate as in the original description.

3 - *Entophlyctis vaucheriae* Fischer 1892 (Plate 3).

Sporangia intramatrical, globose, 27-39μm diam., with a beak-like or narrowly cylindrical apical exit tube more or less prolonged extramatrically, sporangia smooth, thin-walled and colourless. Rhizoids arising basally from a delicate central axis or occasionally from two places in the lower part of

the body, fairly extensive and much branching with tapering ends. Zoospores globose, 6-7μm diam., with a centric or eccentric colourless globule, a single posterior flagellum, emerging upon deliquescence of the papilla and remaining in a compact motionless mass at the orifice before assuming motility. Resting spores globose, up to 34μm diam., with slightly thickened walls, containing numerous oil globules; germinating in the spring by the swelling of the endospore which bursts the exospore, the former emerging as a subglobose structure within which the zoospores are produced. This strain was baited with corn husks.

The Egyptian isolate of *E. vaucheriae* completely agrees with the original description.

B) Sufamily Diplophlyctoideae:

4 - *Diplophlyctis nephrochytrioides* Karling 1967 (Plate 4).

Asexual monocentric thallus, intramatrical, with branched rhizoidal system. Sporangia apophysate, hyaline, smooth, globose to subglobose, up to 34μm diam., irregular, ovoid, 24-51 x 18-40μm, encysted planospores and germ tube remain attached to the zoosporangium. Rhizoids arising from a basal axis, richly branched and extending up to 160μm. Zoospores subglobose 5-6μm diam., with a large hyaline refractive globule. Resting spores globose, 18-21μm diam., with a thick dark brown wall and a large central globule surrounded by smaller ones; apophysis attached to the central rhizoidal axis and bearing rhizoidal filaments at one end. This strain was baited with gromegrasse leaves.

Except for the slightly bigger zoospores in the Egyptian isolate, other morphological features match the original description.

5 - *Diplophlyctis sexualis* Haskins 1950 (Plate 5).

Thalli monocentric, usually intramatrical, consisting of a sporangium or resting spore subtended by an apophysis from which a rather thick-walled, branched rhizoidal system arises. Sporangia variously shaped, hyaline, smooth, up to 30μm diam.; exit tubes one or two, short and broad, dehiscing by deliquescence or by softening of the tips forming an evanescent gelatinous plug, below which is the endo-operculum. Zoospores, 5-5.8μm diam., each with a single refractive globule and a posterior, up to 35μm long flagellum, remaining some time motionless after discharge. Rhizoidal system usually arising from the basal half of the apophysis, stout, extensive and branched. Resting spores globose or slightly oval, 18-22μm diam., contents coarse and globular. This fungus develops well on various cellulolytic substrates, especially cellophane.

Zoospores and resting spores exactly match the original description; sporangia in the Egyptian isolate were slightly smaller than in the original isolate.

6 - *Diplophlyctis verrucosa* Kobayashi & Ookubo 1954 (Plate 6).

Zoosporangia intramatrical, depressed globose, ovoid or ellipsoidal, thin-walled, hyaline, smooth, 27-42 x 16-33μm, with a single apical, basal or

lateral, cylindrical, straight or somewhat curved, 33-87μm long exit tube. Zoospores numerous, hyaline, globose or subglobose, 4.8-6μm diam., with a single refractive globule and a dense cluster of compact granules in one side, with a posterior flagellum; apophysis small; rhizoids basally or bilaterally attached, dichotomously branched. Resting spores globose, up to 28μm diam., with a thick pale yellow wall, containing numerous oil globules; resting spores smooth-walled. This fungus was baited with bromegrasse leaves.

The Egyptian strain of *D. verrucosa* matches the original description except for the absence of roughening of resting spores.

7 - *Nephrochytrium amazonense* Karling 1944 (Plate 7).

Thallus monocentric, usually intramatrical, consisting of a sporangium or resting spore, subtended by an apophysis from which an extensive, richly branched rhizoidal system arises. Sporangia hyaline, smooth, pyriform, 30-120 x 13-18μm, or subglobose, 18-43μm diam., exit tube 10-75μm long; tip of the tube swelling and softening to form a plug of hyaline material; a shallow saucer-shaped operculum subsequently developed inside the exit tube. Zoospores emerging when fully developed and forming a globular mass at the orifice before dispersing; zoospores globose, 6-6.5μm diam., with a large refractive globule, posterior flagellum up to 40μm long. Apophysis oval, flattened, obpyriform, 5-26 x 4-12μm. Rhizoidal system arising from the base of the apophysis, richly branched, up to 156μm long. Resting spores oval, somewhat bean-shaped, 29-42 x 25-35μm, subglobose, 24-28μm diam., and sometime irregular, smooth-walled, functioning as prosporangia on germination. This fungus was baited with onion skin scales.

The Egyptian isolate matches the original description.

8 - *Nephrochytrium appendiculatum* Karling 1938 (Plate 8).

Zoosporangia numerous, intramatrical, hyaline, smooth, subglobose, flattened, dispersed, somewhat kidney-shaped, 33-68 x 8-20μm, with 1-2 exit papillae or tubes of varying length. Zoospores hyaline, globose, 3.5-4.2μm diam., with a large clear refractive globule; posterior flagellum up to 33μm long. Apophysis elongate, transverse, usually spindle-shaped, 15-21 x 6-10μm; rhizoids arising from the end of the apophysis. Resting spores smooth, globose, flattened, occasionally obpyriform, 9-18 x 6-9μm, thick-walled, containing some refractive globules. This fungus was baited with onion skin scales.

The Egyptian isolate perfectly matches the original description.

DISCUSSION

Eight species belonging to four genera of the Entophlyctidaceae were isolated and identified. All were recorded for the first time in Egypt.

The subfamily Entophlyctidoideae includes the genera *Entophlyctis* Fischer which comprises 13 species and *Endochytrium* Sparrow (7 species). Two species of *Entophlyctis*, *E. crenata*, *E. vaucheriae* and one of

Endochytrium, *E. digitatum* were recorded in the present study. Karling (1977) had observed resting spores in *E. crenata* and *E. vaucheriae* and they were also found here. *Endochytrium* is the operculate counterpart of *Entophlyctis*; its species are weakly parasitic in green algae or saprophytic in cellulosic substrata and eggs or cysts of microscopic animals.

The subfamily Diplophlyctidoideae includes the genera *Diplophlyctis* Schroeter (7), *Nephrochytrium* Karling (6) and *Rhizosiphon* Scherffel (3 species). The thallus is monocentric and eucarpic. The enlargment of the germ tube usually becomes an apophysis, which functions as a prosporangium in most species of genera *Diplophlyctis* and *Nephrochytrium* (Karling, 1977). Dogma (1973) proposed two phylogenetically related groups of chytrids, the "rhizophlyctoidal alliance" and the "cladochytrioidal alliance". Because of the comparable degree of natural characters, Hassan (1983) suggested a third one, the "diplophlyctoidal alliance". The core of this alliance may be delimited by the complex of genera *Diplophlyctis* and *Nephrochytrium*.

Diplophlyctis had been characterized as intramatrical, with inoperculate sporangia, but Karling (1977) pointed out that *Diplophlyctis sexualis* appears to be endo-operculate. My observations of *D. sexualis* confirm this. The observation by Haskins (1950), Sparrow (1960) and Karling (1977) that zoosporangium and apophysis develop from a branch of the germ tube in *D. sexualis* was also confirmed in the present study.

Ookubo (1954) observed a fine ornamentation of the resting spore wall in *Diplophlyctis verrucosa*. But, Karling (1977) found that *D. verrucosa* isolated in Iceland was much more regular in shape and growth density, and also the sporangia and resting spores were generally smaller. *D. verrucosa* can parasitize *Chara*; in Iceland, the fungus was found to be saprophytic. The Egyptian isolate in all features matched that from Iceland.

Nephrochytrium has been created by Karling (1938) for a new saprophytic operculate member of Chytridiales occurring on dead or moribund cells of *Chara* and *Nitella*. *N. appendiculatum* was the first species described. Two strains of this genus were recorded in the present study, *N. amazonense* and *N. appendiculatum*. For both species, Karling (1977) illustrated the terminal branches running out to fine filaments and points. Moreover, he added that the resting spores in these two species are borne in the same manner as the sporangium. All these features were observed in the two Egyptian isolates as well.

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Plate 1, Figs. 1-13: *Endochytrium digitatum*. 1: Zoospores; 2-5, 8, 12, 13: Differently shaped zoosporangia; 6: Elongate zoosporangium with curved exit tube; 7: Operculate sporangium with short lateral papilla; 9, 11: Intercalary zoosporangia; 10: Subglobose sporangium with long exit tube.

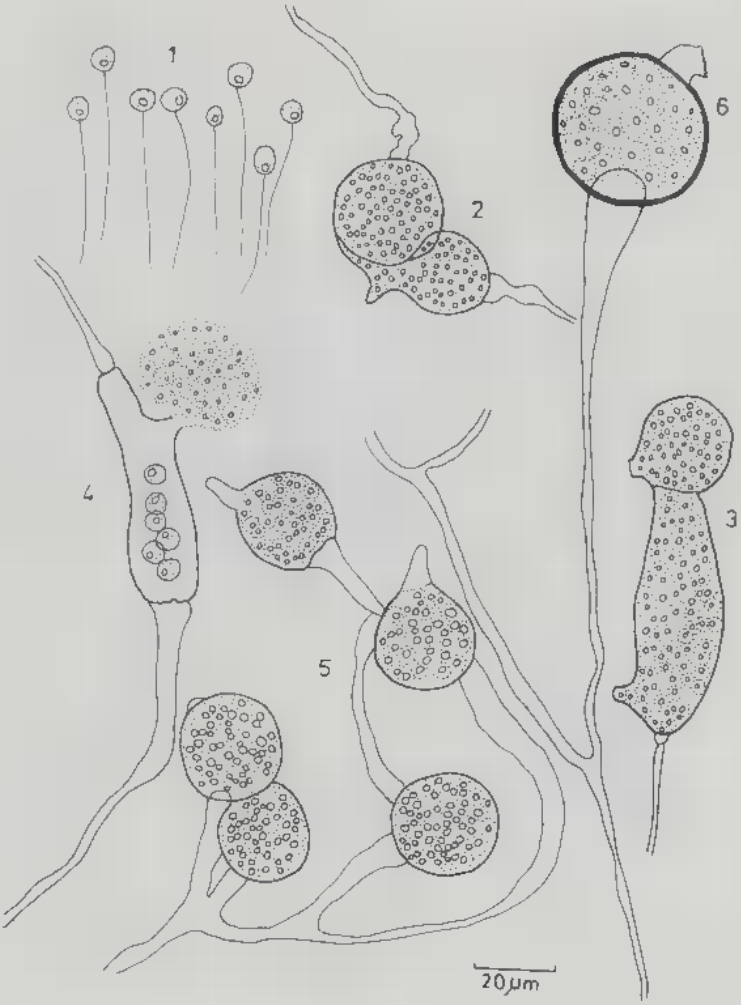

Plate 2, Figs. 1-6: *Entophlyctis crenata*. 1: Zoospores with refractive globule; 2: Intercalary young sporangia; 3: Long cylindrical sporangium with two short papillae; 4: Mature operculate sporangium; 5: Terminal subglobose zoosporangia; 6: Mature resting sporangium with refractive globules.

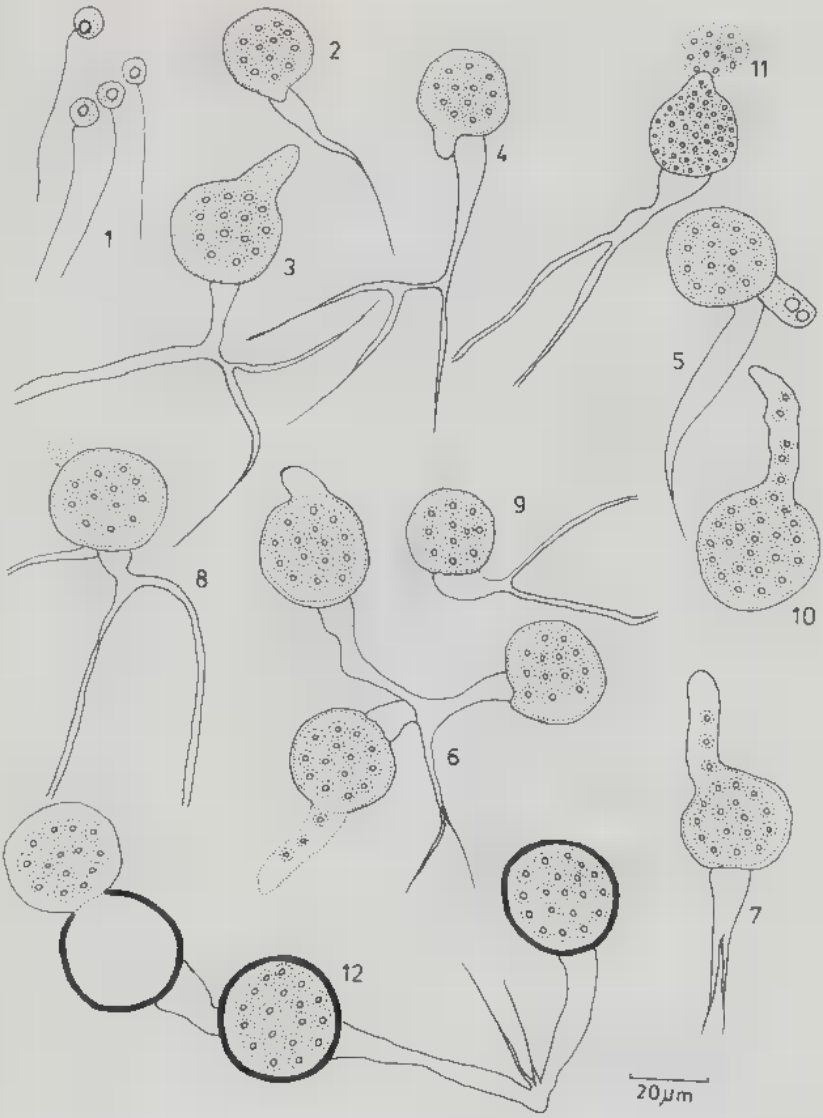

Plate 3, Figs. 1-12: *Entophlyctis vaucheriae*. 1: Zoospores with refractive globule; 2-7: Differently shaped young vacuolate zoosporangia with short tapering rhizoidal axes; 8, 9: Two young sporangia with rhizoidal axis; 10: Sporangium with long exit tube; 11: Operculate zoosporangium; 12: Thick-walled resting spores, one germinating.

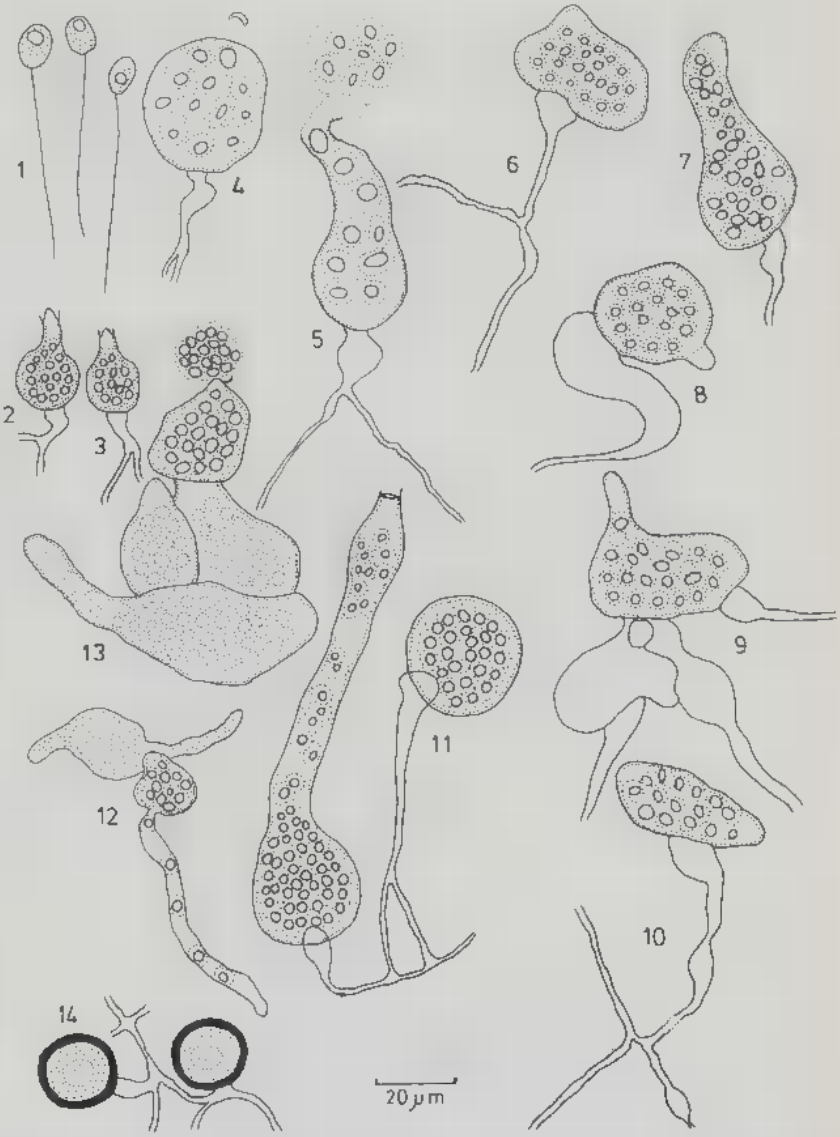

Plate 4, Figs. 1-14: *Diplophlyctis nephrochytrioides*. 1: Zoospores with refractive globule; 2, 3: Young zoosporangia; 4, 5: Mature operculate sporangia; 6-11: Different shapes of apophysate zoosporangia with branched rhizoidal axes; 12, 13: Mature sporangia with germ tube. 14: Resting spores with thick dark-brown wall and central large globule.

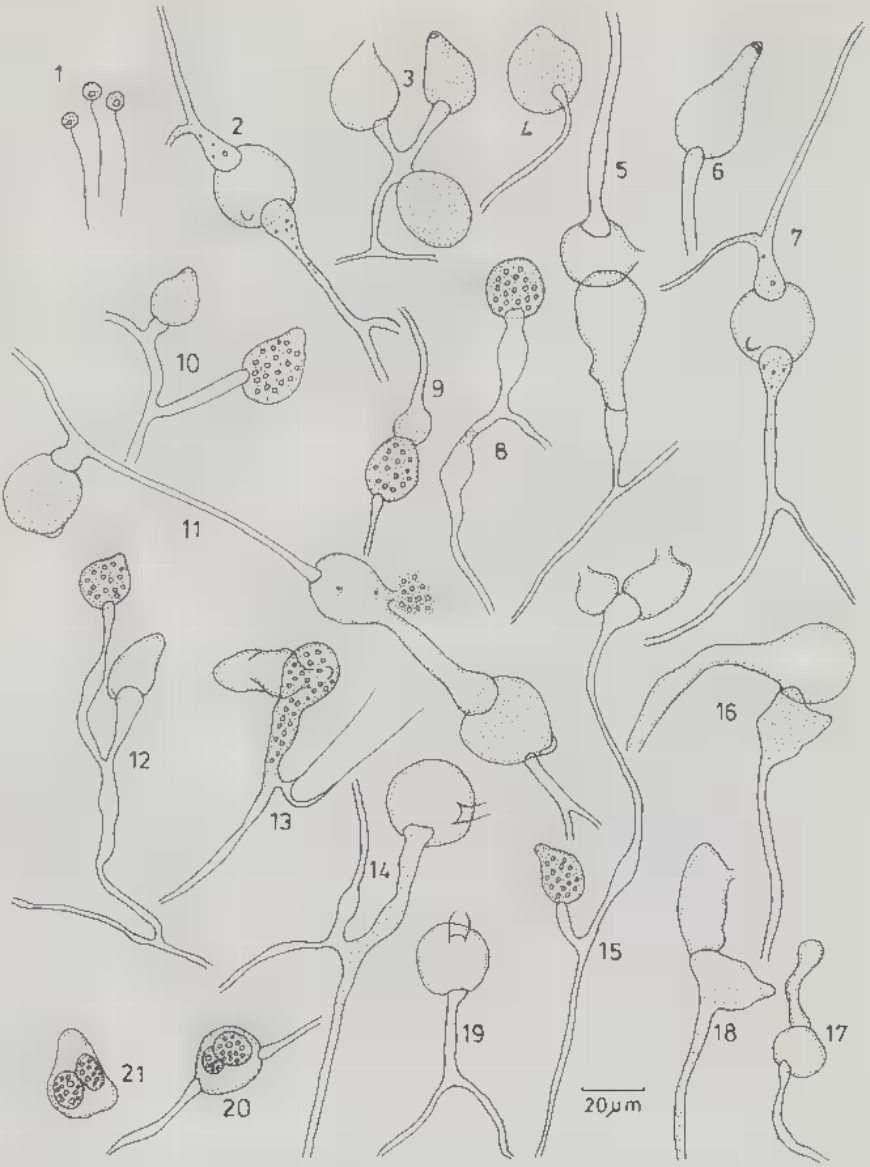

Plate 5, Figs. 1-21: *Diplophlyctis sexualis*. 1: Zoospores; 2-10: Terminal and intercalary apophysate zoosporangia; 11: Mature operculate and young papillate sporangia; 12-19: Empty and young variously shaped sporangia with branched rhizoidal axes; 20, 21: Resting spores of different shapes.

Plate 6, Figs 1-11: *Diplophlyctis verrucosa*. 1: Zoospores with one large and numerous small refractive globules; 2-4: Three young sporangia with long exit tubes and branched rhizoidal axes; 5-8: Young ellipsoidal sporangia; 9: Mature sporangium; 10: Germinating resting spore; 11: Spherical resting spore.

Plate 7, Figs 1-25: *Nephrochytrium amazonense*. 1: Zoospores; 2-6: Young apophysate sporangia; 7-14, 18-22: Mature differently shaped apophysate sporangia; 15, 16: Young and empty apophysate sporangia; 17: Operculate zoosporangium; 23, 24: Differently shaped resting spores; 25: Germinating resting spore.

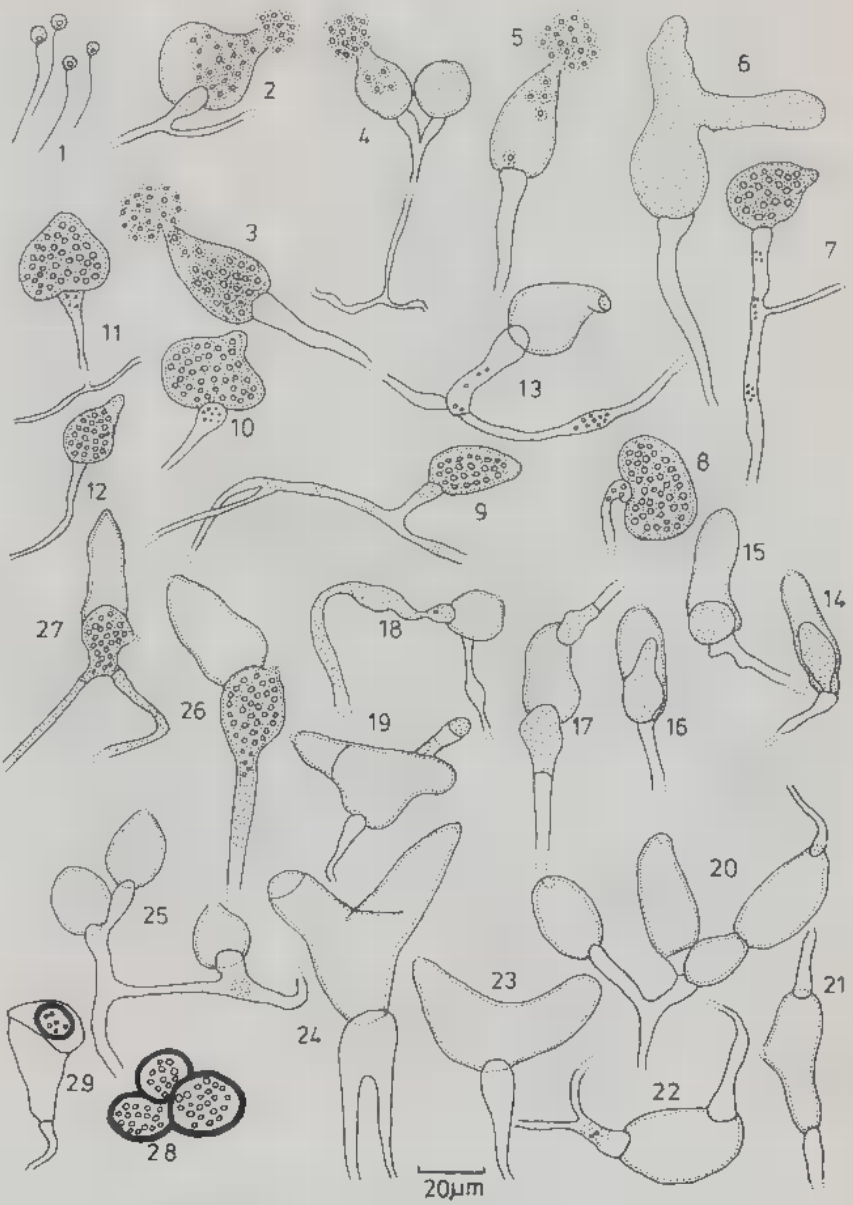

Plate 8, Figs. 1-29: *Nephrochytrium appendiculatum*. 1: Zoospores; 2-5: Operculate zoosporangia; 6: Young sporangium with two exit tubes; 7-12: Differently shaped mature zoosporangia; 13-17, 19-25: Apophysate sporangia; 18: Empty globose sporangium attached with young thallus; 26, 27: Germinating sporangia; 28, 29: Thick-walled resting spores.